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(54) Title: HUMAN VANILLOID RECEPTOR GENE

(57) Abstract: The present invention provides an isolated or purified polynucleotide that encodes human vanilloid receptor. Isoforms of human vanilloid receptor are also disclosed. The invention also provides methods of making recombinant human vanilloid receptor using the polynucleotides and host cells transformed with the polynucleotides.

HUMAN VANILLOID RECEPTOR GENE

RELATED APPLICATIONS

This application claims priority to U.S. Application Serial Number 09/191,139 filed November 13, 1998.

TECHNICAL FIELD

The invention relates generally to polynucleotide sequences and polypeptide sequences encoded therefrom, more specifically, to vanilloid receptor genes and polypeptides encoded therefrom as well as methods which utilize these polypeptides for identifying compounds which modulate vanilloid receptors in human tissues.

BACKGROUND OF THE INVENTION

Vanilloid receptors are a class of ligand-gated ion channels defined by the natural ligands capsaicin, the active ingredient of hot peppers from plants of the genus *Capsium* and resiniferitoxin (RTX), an ultrapotent capsaicin analog found in the latex of *Euphorbia resinifera* (Holzer, Pharmacol. Rev. 43:143-201 [1991]). These receptors are involved in a variety of physiological processes including nociception, inflammation, regulation of body temperature, cardiovascular and bronchial systems, reflex bladder function and gastric mucosal defense mechanisms (Capsaicin in the study of pain, Wood ed., 1993, Academic Press).

The vanilloid receptor has been characterized as a cation permeable ion channel with the permeability of di- and mono-valent cations being $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$ (Bevan and Szolcsanyl, Trends Pharmacol. 11:330-333 [1990]). Activation of neuronal vanilloid receptors by capsaicin results in initial excitation resulting in pain perception while prolonged exposure results in analgesic effects most likely through a desensitization process (Szallasi, Gen. Pharmac. 25:223-243 [1994]). This biphasic response is characteristic for the other physiological responses to capsaicin, first described for thermoregulation in the hypothalamus (Jancsó-Gábor et al., J. Physiol. 208:449-459 [1970]). The rat vanilloid receptor VR1 has been cloned (Caterina *et al.*, Nature **389**:816-824 [1997]).

Because of capsaicin's role in physiological processes, it would be useful to identify compounds, which modulate the activity of a human vanilloid receptor and/or its analogs. However, efforts to identify such compounds have been hampered by the lack of readily available human vanilloid receptors or cell lines expressing the human vanilloid receptor gene for use in screening assays. Although the rat vanilloid receptor is thought to be expressed exclusively in sensory neurons, human sensory neuron tissue is extremely difficult to obtain. Furthermore, no cell lines have been reported which endogenously express the human vanilloid receptor gene. Thus, there is a need for a simple, easy and cost effective means to obtain large quantities of human vanilloid receptors and/or a cell line which expresses such receptors.

The present invention solves this problem by providing reagents, such as human vanilloid receptors, polynucleotides (and polymorphic variants thereof) which encode for human vanilloid receptors and recombinant expression systems for large-scale production of said receptors. The invention also provides methodologies, such as assays, for identifying compounds which modulate the activity of human vanilloid receptors. Thus, the invention provides high throughput screening assays to identify new vanilloid receptor ligands for the treatment of various disease states including neuropathic pain, inflammation, arthritis, rhinitis, pruritus, bladder dysfunction, cluster headache, wound healing and psoriasis.

SUMMARY OF THE INVENTION

The present invention provides an isolated or purified polynucleotide comprising a nucleotide sequence which encodes a human vanilloid receptor and fragments or complements thereof. Preferably, the nucleotide sequence is SEQ ID NO:1 or fragments thereof. More preferably, the nucleotide sequence is SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050. The invention further provides a polynucleotide comprising a nucleotide sequence which encodes a human vanilloid receptor having the sequence of SEQ ID NO:3.

In another aspect, the polynucleotide can be produced by recombinant techniques. A recombinant molecule comprises a nucleotide sequence that encodes a human vanilloid receptor and is contained within an expression vector. The expression vector may be either a prokaryotic or a eukaryotic vector. Preferred expression vectors are pCIneo and pACSG2. In a more preferred embodiment, the nucleotide sequence which encodes a human vanilloid receptor has the sequence SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050.

The present invention further provides a host cell transformed with said vector. The host cell is either a prokaryotic or eukaryotic cell.

The present invention also provides a polypeptide of a human vanilloid receptor or fragments thereof. In a preferred embodiment, the polypeptide has the amino acid sequence SEQ ID NO:3. The polypeptide can be produced by recombinant technology and provided in purified form.

In another aspect, the invention provides a method for producing a polypeptide which contains at least one human vanilloid receptor epitope, wherein the method comprises incubating host cells transformed with an expression vector comprising a nucleotide sequence which encodes a human vanilloid receptor. Preferably, the expression vector comprises a nucleotide sequence having the sequence SEQ ID NO:1 and fragments and complements thereof. More preferably, the nucleotide sequence has the sequence SEQ ID NO:1 from about

nucleotide position 435 to about nucleotide position 3050. Even more preferably, the nucleotide sequence encodes a human vanilloid receptor having sequence SEQ ID NO:3.

In another aspect, the invention provides a method for identifying compounds that modulate vanilloid receptor activity, comprising the steps of: (a) providing a host cell that expresses the vanilloid receptor polypeptide; (b) mixing a test compound with the cell; and (c) measuring either (i) the effect of the test compound on the cell expressing the receptor, or (ii) the binding of the test compound to the cell or to the receptor. The host cell of the method is either a prokaryotic or eukaryotic cell. Preferably in the method, the measurement of step (c)(ii) is performed by measuring a signal generated by a signal-generating compound or by measuring a signal generated by a radiolabeled ion, a fluorescent probe or an electrical current.

In yet another aspect, the invention provides a method for identifying a cytoprotective compound, comprising the steps of: (a) providing a cell that expresses a vanilloid receptor polypeptide or fragment thereof; (b) combining a test compound with the cell; and (c) monitoring the cell or cellular function for an indication of cytotoxicity. The host cell of the method is either a prokaryotic or eukaryotic cell. Preferably, the method comprises providing a cell which has an expression vector comprising a polynucleotide having the nucleotide sequence SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050 operably linked to control sequences that direct the transcription of the polynucleotide whereby the polynucleotide is expressed in a host cell. More preferably, one of the control sequences comprises an inducible promotor . Even more preferably, the cell is maintained in the presence of a substance which minimizes or blocks a cytotoxic effect on the cell.

In yet another aspect, the invention provides a method of treating an individual having a condition associated with vanilloid receptor modulation, comprising administering to the individual an effective amount of a compound that controls the gene expression of vanilloid receptor, in a pharmaceutically acceptable excipient.

In yet another embodiment, the invention provides a monoclonal antibody or a polyclonal antibody which specifically binds to human vanilloid receptor having amino acid sequence SEQ ID NO:3 or fragments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the alignment (default parameters of the FRAMEALIGN Program, Wisconsin Sequence Analysis Package, Version 9, Genetics Computer Group, Madison, WI) between the consensus sequence (upper line) of the overlapping human Incyte ESTs 1427917 (nt 1-227) and 3460342 (nt 32-270) with the corresponding amino acid sequence of the rat vanilloid receptor (bottom line, Caterina et al., 1997, *supra*). In this Figure, the upper line

[redacted]

corresponds to nucleotides 1696-1966 of SEQ ID NO:7. The bottom line corresponds to amino acid residues 500-589 of SEQ ID NO:4.

FIG. 2 shows the alignment (default parameters of the GAP Program, Wisconsin Sequence Analysis Package, Version 9, Genetics Computer Group, Madison, WI) between cDNA sequences of the human vanilloid receptor (hVR1, top line, SEQ ID NO:7) and rat vanilloid receptor 1 (rVR1, bottom line, SEQ ID NO:2, Caterina et al., 1997, *supra*). Vertical lines between the two sequences indicate identical nucleotides at those positions. Methionine initiation codons (ATG) and stop codons (TGA in top strand and TAA in bottom strand) are boxed. The DNA sequence identity of the rat cDNA from nucleotides (nt) 44-2730 and the human cDNA from nt 163-2874 is 82%.

FIG. 3 shows the multiple alignment (default parameters of the Pileup and Pretty Programs, Wisconsin Sequence Analysis Package, Version 10, Genetics Computer Group, Madison, WI) of the amino acid sequences of the hVR1 (SEQ ID NO:8), rVH1 (SEQ ID NO:4, Caterina et al., 1997, *supra*), human vanilloid receptor-like protein (hVR2, Caterina et al., Nature 398:436-441 1999, SEQ ID NO:15) and human vanilloid receptor 3 (hVR3, SEQ ID NO:3). The consensus sequence identifies any identical amino acid position shared by these 4 proteins. Boxed regions indicate the ankaryn repeats (position 239-270, 294-317 and 370-403), potential transmembrane domains (positions 471-493, 519-540, 555-574, 579-597, 621-640 and 703-730) and the poor-loop region (position 671-691).

FIG. 4 shows the polymorphic regions of the human vanilloid receptor determined by direct sequencing of the PCR product of human small intestine RNA. The Sequencher™ chromatogram tracings (Sequencher™ Version 3.0, Gene Codes Corp., Ann Arbor, MI) are shown with arrows identifying the double peaks consistent with polymorphic positions. Nt position 1605 contains either a C or a T while nt position 1952 contains an A or a G.

FIG. 5 shows the GAP analysis of hVR1 (bottom sequence, positions 301-3410 of SEQ ID NO:7) and hVR3 (top sequence, positions 1-3055 of SEQ ID NO:1) DNA sequences.

FIG. 6 shows the GAP analysis of the derived amino acid sequences of hVR1 (bottom sequence) and hVR3 (top sequence), SEQ ID NOs:8 and 3 respectively.

FIG. 7 shows a graphical representation of expression of hVR1 and hVR3 by quantitative RT-PCR (ABI Prism 7700) of total RNA isolated from human adrenal gland (lane 1), brain (lane 2), cerebellum (lane 3), fetal brain (lane 4), fetal liver (lane 5), heart (lane 6), kidney (lane 7), liver (lane 8), lung (lane 9), mammary gland (lane 10), pancreas (lane 11), placenta (lane 12), prostate (lane 13), salivary gland (lane 14), skeletal muscle (lane 15), small intestine (lane 16), spleen (lane 17), stomach (lane 18), testes (lane 19), thymus (lane 20), trachea (lane 21), uterus (lane 22), DRG (lane 23), bladder (lane 24) and HEK293 cells (lane 25) using primers specific for hVR1 and hVR3. The hatched bars represent samples from an additional experiment.

FIG. 8 shows the nucleotide sequence (SEQ ID NO:1) of human vanilloid receptor 3 and deduced amino acid sequence (SEQ ID NO:3).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated and purified polynucleotides that encode a human vanilloid receptor, fragments thereof, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process for making a human vanilloid receptor using those polynucleotides and vectors, and isolated and purified recombinant human vanilloid receptor and polypeptide fragments thereof.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain cDNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, targets for pharmaceutical screening assays and/or as components or target sites for various therapies. Isolation of sequences from other portions of the vanilloid receptor gene can be accomplished by utilizing probes or PCR primers derived from these nucleic acid sequences, thus allowing additional probes and polypeptides of the genome of interest to be established.

The present invention also provides methods for assaying a test sample for products of a human vanilloid receptor gene, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of a human vanilloid receptor gene. The method may include an amplification step, wherein portions of the cDNA corresponding to the gene or fragment thereof is amplified. Methods also are provided for assaying for the translation products of mRNAs. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods. For example, the invention provides monoclonal and polyclonal antibodies directed against at least one epitope contained within the polypeptide sequences of the invention which are useful for diagnostic tests and for screening for diseases or conditions associated with abnormal vanilloid receptor production.

Although the physiological manifestations of abnormal vanilloid receptor expression are as yet unknown in humans or other mammals, we postulate that the vanilloid receptor may play a pathological role resulting from its abnormal expression. For example, Caterina et al. have shown that HEK293 cells transfected with the vanilloid receptor are killed within several hours of continuous exposure to capsaicin. Therefore, it is reasonable to postulate that the presence of

vanilloid receptor in certain body fluids where it is not normally found may be indicative of a disease state, the further progression of which could be monitored by assaying for vanilloid receptor in such fluids. A similar role is seen for myelin basic protein (MBP) which in the normal physiological state is a membrane bound protein and therefore not found in body fluids, but in disease states such as multiple sclerosis, it is released into cerebral spinal fluid. Furthermore, the presence of a polynucleotide or fragment thereof which encodes vanilloid receptor in tissues or body fluids where it is unexpected, may also be indicative of a disease condition, in the case, for example, where the disease was manifest by cellular degeneration.

Thus, the reagents and methods described herein may enable the identification of certain markers as indicative of abnormal vanilloid receptor expression and the information obtained therefrom may aid in the diagnosis, staging, monitoring, prognosis and/or therapy of diseases or conditions which may be associated with such expression. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR); and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which an immunogenic epitope may be found. Preferably, such an epitope is unique to the disease state or condition associated with the vanilloid receptor gene. The uniqueness of the epitope may be determined by its immunological reactivity with the polypeptide product encoded by such gene, and lack of immunological reactivity with tissue(s) from non-diseased patients. Methods for determining immunological reactivity are well-known and include but are not limited to, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA); chemiluminescent immunoassay (CLIA), and others; several examples of suitable methods are described herein.

Definitions

All patents, patent applications and publications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the content clearly dictates otherwise.

Unless otherwise stated, the following terms shall have the following meanings:

"Purified product" refers to a preparation of the product, which has been isolated from the cellular constituents with which the product is normally associated, and from other types of cells, which may be present in the sample of interest.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same

polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such a polynucleotide could be part of a vector and/or such a polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as, double- and single-stranded RNA. It also includes modifications, such as methylation or capping, and unmodified forms of the polynucleotide.

A "vanilloid receptor variant" refers to an isolated vanilloid receptor polynucleotide sequence having at least 83%, more preferably, at least 90% and even more preferably, at least 95% global sequence identity over a length of a vanilloid receptor polynucleotide, to vanilloid receptor polynucleotides disclosed herein. "Percent identity" is determined using the default parameters of the GAP program, Wisconsin Sequence Analysis Package, Version 9, Genetics Computer Group, Madison, WI).

A "polynucleotide fragment derived from" a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, is preferably at least about 8 nucleotides, is more preferably at least about 10, is more preferably at least about 12 nucleotides, is more preferably at least about 15 and even more preferably is at least about 20 nucleotides corresponding, i.e., identical to or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary to or identical to a sequence which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to surmise the uniqueness of a designated sequence. Regions from which sequences may be derived include but are not limited to regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including but not limited to chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived; as such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

When referring to a nucleic acid fragment, such a fragment is considered to "selectively hybridize" or to "selectively bind" to a polynucleotide or variants thereof disclosed herein, if, within the linear range of detection, the hybridization results in a

stronger signal relative to the signal that results from hybridization of the fragment to an equal amount of a second polynucleotide. A signal which is "stronger" than another is one which is measurable over the other by the particular method of detection. Methods for hybridizing and detecting polynucleotides are well known to those of ordinary skill in the art.

Also, when referring to a nucleic acid fragment, such a fragment is considered to hybridize under selective hybridization conditions if it selectively hybridizes under (i) typical hybridization and wash conditions, such as those described, for example, in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, second edition, (1989), Cold Spring Harbor, N.Y. and Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington D.C.; IRL Press), where preferred hybridization conditions are those of lesser stringency and more preferred, higher stringency; or (ii) standard PCR conditions (Saiki, R.K. *et al.* (1988) Science. 239:487-491) or "touch-down" PCR conditions (Roux, K.H., (1994), Biotechiques, 16:812-814).

"A sequence corresponding to a cDNA" means that the sequence contains a polynucleotide sequence that is identical to or complementary to a sequence in the designated DNA. The degree (or "percent") of identity or complementarity to the cDNA will be approximately 50% or greater, will preferably be at least about 70% or greater, and more preferably will be at least about 90% or greater. The sequence that corresponds will be at least about 50 nucleotides in length, will preferably be about 60 nucleotides in length, and more preferably, will be at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art, and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90% of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density. Thus, "purified polypeptide" means a polypeptide of interest or fragment thereof which is essentially free, that is, contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90%

of cellular components with which the polypeptide of interest is naturally associated. Methods for purifying are known in the art.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, i.e., peptide nucleic acid analog (PNA) or morpholino analog (MA) which can be used to identify specific DNA or RNA present in samples bearing the complementary sequence.

The term "primer" denotes a specific oligonucleotide sequence complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence and serve as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase or reverse transcriptase.

"Polypeptide" as used herein indicates a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, oligopeptides and proteins are included within the definition of polypeptide. This term, however, is not intended to refer to post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like.

A "recombinant polypeptide" as used herein means at least a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it is linked in nature. A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to those of ordinary skill in the art. These synthetic peptides are useful in various applications.

A vanilloid receptor polypeptide, as used herein, refers to polypeptide having at least 87%, more preferably at least 90%, and even more preferably at least 95% global sequence identity over a length of a vanilloid receptor polypeptide, to vanilloid receptor polypeptides disclosed herein. A most preferred vanilloid receptor polypeptide is SEQ ID NO:3. Two other preferred vanilloid receptor polypeptides have essentially identical sequences to SEQ ID NO:3 with the exception that in one preferred polypeptide, at residue 469, threonine is replaced by isoleucine and in the second preferred polypeptide, at residue 586, isoleucine is replaced with valine.

A "polypeptide or amino acid sequence derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence or a portion thereof wherein the portion consists of at least 3 to 5 amino acids, and more preferably at least 8 to 10 amino acids, and even more preferably 15 to 20 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

As used herein, the term "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. The term "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" can be determined between the compared polypeptide sequences. Further, the polypeptide or amino acid sequence may preferably have at least 90% similarity, more preferably about 95% similarity and most preferably about 98% similarity to a polypeptide or amino acid sequence of a human vanilloid receptor.

The percent identity of two sequences, whether nucleic acid or peptide sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Schwartz, R.M., and Dayhoff, M.O. Matrices for detecting distant relationships, (in) Atlas of Protein Sequence and Structure, 5 suppl.3:353-358, (Nat. Biomed. Res. Found., Washington D.C.), 1978. and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their GAP utility application. The default parameters for this method are described in the Wisconsin Sequence analysis Package, Program Manual, Version 9 (available from Genetics Computer Group, Madison, WI). Other equally suitable programs for calculating the percent identity or similarity between sequences are generally known in the art.

Other techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded therein, and comparing this to a second amino acid sequence.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original progeny of the original cell which has been transfected.

As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoter, ribosomal binding site and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and recombinant polynucleotide sequences.

The term "sense strand" or "plus strand" (or "+") as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term "antisense strand" or "minus strand" (or "-") denotes a nucleic acid that contains a sequence that is complementary to that of the "plus" strand.

An "Expressed Sequence Tag" or "EST" refers to the partial sequence of a cDNA insert which has been made by reverse transcription of mRNA extracted from a tissue, followed by insertion into a vector.

A "transcript image" refers to a table or list giving the quantitative distribution of ESTs in a library and represents the genes active in the tissue from which the library was made.

The term "immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to those of ordinary skill in the art and also are described herein. The uniqueness of an epitope also can be surmised by computer searches of known data banks, such as GenBank, for the polynucleotide sequences which encode the epitope, and by amino acid sequence comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of specific juxtaposition of amino acids in an immunologically recognizable structure, such amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide containing an epitope of interest" means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

The terms "transformation" refers to the insertion of an exogenous polynucleotide into a prokaryotic or yeast host cell, irrespective of the method used for the insertion. Generally, the term "transfection" is used with respect to insertion of an exogenous polynucleotide into a eukaryotic host cell. The processes for achieving transformation and/or transfection are well known to those of ordinary skill in the art and include such techniques as direct uptake, transduction, f-mating and electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" refers to prophylaxis and/or therapy.

The term "individual" as used herein refers to vertebrates, particularly members of the mammalian species and includes but is not limited to domestic animals, sports animals, primates and humans; more particularly the term refers to humans.

The term "test sample" refers to a component of an individual's body which is the source of the analyte (also referred to "target" or "marker"). These components include antibodies and antigens and are well known in the art. These test samples include biological samples which can be tested by the methods of the present invention described herein and

include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitor-urinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; fixed tissue specimens; and fixed cell specimens.

"PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with such signal generating compounds as fluorescein, radionucleotides, chemiluminescent compounds, and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routineer that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

"Analyte," as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a peptide, an amino acid, a nucleotide target, and the like.

The present invention provides assays which utilize specific binding members. A "specific binding member," as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like.

Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal, and complexes thereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent," as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. The indicator reagent can be a member of any specific binding pair including hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazol or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

"Solid phases" ("solid supports") are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, and

Duracytes® (red blood cells "fixed" by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structure generally are preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include but are not limited to nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1mm. The pore size may vary within wide limits, and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

Reagents

The present invention provides reagents such as polynucleotide sequences derived from a human vanilloid receptor gene, polypeptides encoded therein, and antibodies produced from these polypeptides. The present invention also provides reagents such as oligonucleotide

fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. For example, selected vanilloid receptor-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression.

The present invention also provides methods, in particular, recombinant methodologies using polynucleotide sequences disclosed herein, for making human vanilloid receptors in high yield as well as methods to identify compounds which modulate (i.e. activate or repress) the activity of such receptors. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably purified.

Furthermore, the polynucleotides disclosed herein, their complementary sequences or fragments of either can be used in assays to detect, amplify or quantify genes, cDNAs or mRNAs encoding human vanilloid receptor. They also can be used to identify an entire or partial coding region which encodes for a vanilloid receptor polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide(s) may be in the form of mRNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding (sense) strand or non-coding (antisense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

In addition, the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence

is a preprotein and may have the leader sequence cleaved by the host cell to form the form of the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional 5' amino acid residues. A protein having a prosequence is a proprotein and may in some cases be an inactive form of the protein. Once the prosequence is cleaved an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence or for a protein having both a presequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pProEx1 (Life Technologies, Gaithersburg, MD) vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson, *et al.*, Cell 37:767 (1984).

It is contemplated that polynucleotides which encode a human vanilloid receptor will be considered to hybridize to the sequences provided herein if there is at least 60%, more preferably at least 70% and even more preferably at least 80%, identity between the polynucleotide and the sequence.

The present invention further provides human vanilloid receptor polypeptides which have the deduced amino acid sequences as provided herein, as well as fragments, analogs and derivatives of such polypeptides. The polypeptides of the present invention may be recombinant polypeptides, natural purified polypeptides or synthetic polypeptides. The polypeptides, fragments, derivatives or analogs of the human vanilloid receptor may be those in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or

threonine with serine. In contrast, variations may include nonconservative changes, eg, replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison, WI).

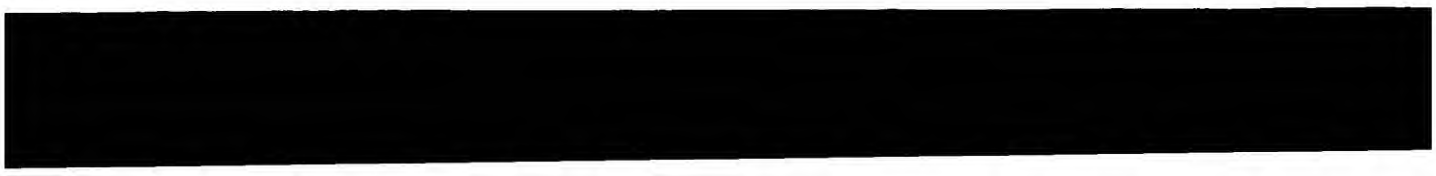
A human vanilloid polypeptide is believed to be substantially encoded by or within SEQ ID NO:1 or SEQ ID NO:7. The minimum polypeptide sequence necessary for ligand binding, however, can be determined by routine methods. The sequence, for example, may be truncated at either end by treating an appropriate expression vector with an exonuclease (after cleavage at the 5' or 3' end of the coding sequence) to remove any desired number of base pairs. The resulting coding polynucleotide is then expressed and the sequence determined. In this manner the binding activity may be correlated with the amino acid sequence: a limited series of such experiments (removing progressively greater numbers of base pairs) determines the minimum internal sequence necessary for ligand-binding activity.

The vanilloid receptor polypeptides may be naturally purified products expressed from a high expressing cell line, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture) as described above. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers or produced by cell-free translation systems using RNAs derived from the DNA constructs of the present invention.

The present invention also provides an antibody produced by using a purified vanilloid receptor gene polypeptide of which at least a portion of the polypeptide is encoded by a vanilloid receptor gene polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of vanilloid receptor polypeptides in test samples. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of a vanilloid receptor polypeptide in conditions associated with its altered or abnormal expression. The antibody may also be used to detect an accessory protein or proteins by immunoprecipitation of protein complexes.

Probe Assays

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. For example, such probes can be used



in Fluorescent In Situ Hybridization (FISH) technology to perform chromosomal analysis, and used to identify vanilloid receptor structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotide probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly- detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in fixed tissue specimens or cells.

The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multigene family or in related species like mouse and man.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize at the outside ends of complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then hybridize to the target sequences and are extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. patents 4,683,195 and 4,683,202.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to a target. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more

completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A-439 182 to K. Backman *et al.*, published July 31, 1991, both of which are incorporated herein by reference.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770, or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, *et al.*, *PCR Methods and Applications* 4: 80-84 (1994).

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described in *PNAS USA* 87:1874-1878 (1990) and also described in *Nature* 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G. T. Walker *et al.*, *Clin. Chem.* 42:9-13 (1996)) and European Patent Application No. 684315; and target mediated amplification, as described by PCT Publication WO 9322461.

In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method herein provided are labeled with capture and detection labels wherein probes are labeled with one type of label and primers are labeled with the other type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (an amplicon) are produced. In the usual case, the amplicon is double stranded because primers are provided to amplify a target sequence and its complementary strand. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

As the single stranded amplicon sequences and probe sequences are cooled, the probe sequences preferentially bind the single stranded amplicon members. This finding is counterintuitive given that the probe sequences are generally selected to be shorter than the primer sequences and therefore have a lower melt temperature than the primers. Accordingly, the melt temperature of the amplicon produced by the primers should also have a higher melt temperature than the probes. Thus, as the mixture is cooled, the re-formation of the double stranded amplicon is expected. As previously stated, however, this is not the case. Probes have been found to preferentially bind the single stranded amplicon members. Moreover, this

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preference of probe/single stranded amplicon binding exists even when the primer sequences are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained therein to release target nucleic acids. The target sequence is either double stranded or single stranded. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of which are complementary to a target sequence and two of which are similarly complementary to the targets complement, are generally employed. While the amplification primers initiate amplification of the target sequence, in some cases, the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example, peptide nucleic acids which are disclosed in International Patent Application WO 92/20702; morpholino analogs which are described in U.S. Patents Nos 5,185,444, 5,034,506, and 5,142,047; and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendable" in that additional dNTPs cannot be added to the probe. In and of themselves analogs usually are non-extendable and nucleic acid probes can be rendered non-extendable by modifying the 3' end of the probe such that the hydroxyl group is no longer

capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 describes modifications which can be used to render a probe non-extendable.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long.

Alternatively, a probe may be involved in the amplifying a target sequence, via a process known as "nested PCR". In nested PCR, the probe has characteristics, which are similar to those of the first and second primers normally used for amplification (such as length, melting temperature etc.), and as such, may itself serve as a primer in an amplification reaction. Generally in nested PCR, a first pair of primers (P_1 and P_2) are employed to form primary extension products. One of the primary primers (for example, P_1) may optionally be a capture primer (i.e. linked to a member of a first reactive pair), whereas the other primary primer (P_2) is not. A secondary extension product is then formed using a probe (P_1') and a probe (P_2') which may also have a capture type label (such as a member of a second reactive pair) or a detection label at its 5' end. The probes are complementary to and hybridize at a site on the template near or adjacent the site where the 3' termini of P_1 and P_2 would hybridize if still in solution. Alternatively, a secondary extension product can be formed using the P_1 primer with the probe (P_2') or the P_2 primer with the probe (P_1') sometimes referred to as "hemi-nested PCR". Thus, a labeled primer/probe set generates a secondary product which is shorter than the primary extension product. Furthermore, the secondary product may be detected either on the basis of its size or via its labeled ends (by detection methodologies well known to those of ordinary skill in the art). In this process, probe and primers are generally employed in equivalent concentrations.

Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is a matter of routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), Dupont (Wilmington, DE), or Milligen (Bedford, MA). Many methods have been described for labeling oligonucleotides such as

the primers or probes of the present invention. Enzo Biochemical (New York, NY) and CLONTECH (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (CLONTECH). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (CLONTECH). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications US. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, teach methods for labeling probes at their 5' and 3' termini, respectively. Publications WO92/10505, published 25 June 1992 and WO 92/11388 published 9 July 1992 teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong *et al.*, Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen *et al.*, published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

Capture labels are carried by the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood, of course that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides wherein at least one polynucleotide is provided herein, hybridizing the test sample with the plurality of polynucleotides and detecting the hybridization complexes. The hybridization complexes are identified and quantified to compile a profile which is indicative of vanilloid receptor expression. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

Drug Screening and Gene Therapy.